

Experimental Resin Cements Containing Bioactive Fillers Reduce Matrix Metalloproteinase-mediated Dentin Collagen Degradation

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Abstract

Introduction: Collagen dentin matrix may represent a suitable scaffold to be remineralized in the presence of bioactive materials. The purpose of this study was to determine if experimental resin cements containing bioactive fillers may modulate matrix metalloproteinase-mediated collagen degradation of etched dentin.

Methods: Human dentin beams demineralized using 10% phosphoric acid or 0.5 mol/L EDTA were infiltrated with the following experimental resins: (1) unfilled resin, (2) resin with Bioglass 45S5 particles (Sylc; OSsray Ltd, London, UK), and (3) resin with β -tricalcium phosphate-modified calcium silicate cement (HCAT- β) particles. The filler/resin ratio was 40/60 wt%. The specimens were stored in artificial saliva, and the determination of C-terminal telopeptide (ICTP) was performed by radioimmunoassay after 24 hours, 1 week, and 4 weeks. Scanning electron microscopic analysis of dentin surfaces after 4 weeks of storage was also executed.

Results: Collagen degradation was prominent both in phosphoric acid and EDTA-treated dentin. Resin infiltration strongly reduced the MMP activity in demineralized dentin. Resin-containing Bioglass 45S5 particles exerted higher and more stable protection of collagen at all tested dentin states and time points. HCAT- β induced collagen protection from MMPs only in EDTA-treated specimens. Dentin remineralization was achieved when dentin was infiltrated with the resin cements containing bioactive fillers. **Conclusions:** MMP degradation of dentin collagen is strongly reduced in resin-infiltrated dentin. The inclusion of Bioglass 45S5 particles exerted an additional protection of collagen during dentin remineralization. (*J Endod* 2012;38:1227–1232)

Key Words

Bioactive filler, bioglass, calcium phosphate, collagen, demineralization, dentin, EDTA, metalloproteinases, phosphoric acid, remineralization

The clinical success of resin adhesion to dentin is undermined because of the loss of integrity of the resin-/dentin-bonded interfaces (1). A demand for engineered dentin tissue is growing. For this purpose, 3-dimensional scaffolds are needed to provide a structural support for further remineralization. Partial acid demineralized collagen dentin matrix may represent a suitable scaffold to be remineralized in the presence of bioactive materials (2). However, demineralized exposed collagen, which is unprotected by resin or intrafibrillar minerals, can undergo degradation by endogenous matrix metalloproteinases (MMPs) (3). MMPs are present in radicular dentin (4), and MMP-mediated collagen degradation has also been described in phosphoric acid (PA) and EDTA-treated dentin (5). Effective inhibitors of MMPs within the resin-dentin interfaces may protect the crystallite-sparse collagen fibrils of the scaffold from degradation before they can be remineralized (2).

Biodegradable hydrophilic polymers and bioactive silicates have been proposed as suitable materials for remineralizing scaffolds (6). The use of hydrophilic monomers may also inhibit MMP-mediated collagen degradation through the adsorption of MMPs (7). The coordination of the hydroxyl group of hydroxyethyl methacrylate (HEMA) with Zn^{2+} that is present in the catalytic domain of MMPs has been suggested as an inhibitory mechanism (8). However, resins used in dental adhesives because of their high hydrophilicity and their low degree of cure are not stable and are hydrolyzed with time (1). Because of the chemical similarity to the inorganic phase of dentin, biomaterials such as calcium/sodium phosphosilicate (Bioglass 45S5 [BAG]), tricalcium silicates, and tricalcium phosphate (TCP) have also been investigated in respect to their application in tissue engineering (9). These materials may also be applied in endodontics and restorative dentistry including root-end filling, root perforation repair, and apex completion (10, 11).

A number of recent studies have shown that resin cements filled with bioactive particles may possess bioactivity properties when immersed in phosphate-based solutions (ie, simulated body fluid) to induce apatite formation (10, 11). However, there is a lack of information on the relationship between the remineralization activity of these cements and MMP action on dentin collagen.

In the present study, the effect of resin cements filled with bioactive particles on MMP activity was investigated for the first time. Two experimental cements designed for root-end and root repair filling materials were prepared. BAG and β -TCP-modified calcium silicate cements (HCATs- β) were incorporated as bioactive microfillers in resin-based materials.

The aim of the present study was to ascertain if the combination of a monomer mixture (hydrophobic and hydrophilic resins) filled with 2 different bioactive particles

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may inhibit MMP-mediated collagen degradation of acid-treated human dentin. The null hypothesis to be tested was that MMP-mediated dentin collagen degradation is not decreased when demineralized dentin is infiltrated with resin filled with bioactive particles.

Materials and Methods

Preparation of Experimental Adhesives

Hydrophobic monomers (UDMA/Bis-GMA/TEGDMA; Esstech, Essington, PA) were used to formulate the basic resin comonomer blend with a ratio of 30/10/20 wt%. A hydrophilic monomer (HEMA) (20–40 wt%; Aldrich Chemical Co, Gillingham, UK), absolute ethanol (20–40 wt%), camphoroquinone (1.0 wt%), and ethyl-4-dimethylaminobenzoate (1.0 wt%) (Aldrich Chemical Co) were also added to the resin blend. Five minutes of sonication (Model QS3, Ultrawave Ltd, Cardiff, UK) and 2 days of shaking (Orbital Shakers PSU-20i; Cole Fisher Scientific Ltd, Loughborough, UK) were required to yield well-mixed resin mixtures.

Two different fillers were incorporated (a filler/resin ratio of 40/60 wt%) to formulate experimental endodontic resin cements: (1) BAG with particle sizes ranging between 1 and 20 μm (Sylc; OSspray Ltd, London, UK) and (2) an experimental HCAT- β . The HCAT- β was obtained by mixing 10wt% β -TCP ($\text{Ca}_3[\text{PO}_4]_2$), deionized water (ratio 2:1), and 90wt% of type I ordinary Portland cement (Italcementi Group, Cesena, Italy), which is made up of alite ($3\text{CaO} \times \text{SiO}_2$), belite ($2\text{CaO} \times \text{SiO}_2$), tricalcium aluminate ($3\text{CaO} \times \text{Al}_2\text{O}_3$), and gypsum ($\text{CaSO}_4 \times 2\text{H}_2\text{O}$) (12). Subsequent to the setting time (24h/ $\sim 21^\circ\text{C}$), the cement was incubated in a furnace at 40°C for 12 hours, milled, and sieved to obtain $<20\text{-}\mu\text{m}$ particles.

Three experimental resins were subsequently formulated: (1) resin A: 60 wt% resin blend/40 wt% HCAT- β , (2) resin B: 60 wt% resin blend/40 wt% BAG, and (3) resin C: 100% resin blend (no filler). Fillers were washed previously with a solution of 5% polyacrylic acid to reduce the chemical curing effects induced by the oxides constituting the mineral structure of the fillers. Each resin formulation was then funneled into dark plastic bottles and stored until they were used.

Dentin-Resin Beam Preparation and C-Terminal Telopeptide of Type I Collagen Determinations

Ten sound unerupted human third molars were extracted with informed consent from 5 donors (younger than 25 years of age) under a protocol approved by the institutional review board. The teeth were stored in 0.01% (w/v) thymol solution at 4°C . A dentin disk (0.75 ± 0.08 mm thick) was sectioned from the midcoronal portion of each tooth. Four to 5 dentin beams ($0.75 \times 0.75 \times 5.0$ mm) were obtained from each dentin disk as previously described by Osorio et al (7).

One third of the obtained dentin beams remained untreated, whereas the other two thirds were demineralized in 10% PA ($\text{pH} = 1.0$) (Panreac Química, Barcelona, Spain) for 12 hours at 25°C or demineralized in 0.5 mol/L EDTA ($\text{pH} = 7.4$) (Panreac Química) for 6 days at 25°C . Dentin beams were rinsed in deionized water under constant stirring at 4°C for 72 hours to remove PA and EDTA remnants. Untreated, PA-etched and EDTA-treated dentin beams were then distributed in the following groups: (1) no resin infiltration, (2) neat resin infiltration, (3) HCAT- β -filled resin infiltration, and (4) BAG-filled resin infiltration. For resin infiltration, beams were immersed in the different resin mixtures for 12 hours. The demineralization and resin immersion processes were performed at 25°C in the dark. After removing beams from resins, polymerization was performed. The samples were irradiated in different positions for 40 seconds until the entire area was exposed. The light was tested for light output

(600 mW/cm²) by means of a Demetron radiometer (Model 100; Demetron Research Corp, Danbury, CT).

Dentin beams were rinsed in deionized water under constant stirring at 4°C for 12 hours and then dried for 8 hours in anhydrous calcium sulphate (Sigma-Aldrich, St Louis, MO). The dry mass of each dentin beam was measured with a digital microbalance (accuracy: 0.01 mg) (Analytical Digital Microbalance Model HM-202; A&D Engineering Inc, San Jose, CA). Specimens were rehydrated in 0.9% NaCl (Sigma-Aldrich) containing 10 U/mL penicillin G (Sigma-Aldrich) and 300 $\mu\text{g/mL}$ streptomycin ($\text{pH} = 7.0$, Sigma-Aldrich) for 24 hours. Two dentin beams were placed in Eppendorf tubes containing artificial saliva, 50 mmol/L HEPES (Applchem, Darmstadt, Germany), 5 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Panreac Química), 0.001 mmol/L ZnCl_2 (Sigma-Aldrich), 150 mmol/L NaCl, 100 U/mL penicillin (Sigma-Aldrich), 1,000 $\mu\text{g/mL}$, and streptomycin ($\text{pH} = 7.2$, Sigma-Aldrich) (7).

Dentin specimens were incubated in 500 μL media at 37°C for 4 weeks. After 24-hour, 1-week, and 4-week periods, sample volumes of 100 μL media were withdrawn after agitation in a Vortex Mixer (Velp Scientifica SRL, Lab Solutions, Milan, Italy) at 800 rpm during 2 minutes. Media was not replaced at any time. These samples were analyzed for the liberation of collagen degradation product (C-terminal telopeptide of type I collagen [ICTP]) using a radioimmunoassay kit (ICTP-RIA Cat. No. 68601; Orion Diagnostica Oy, Espoo, Finland). A standard curve was constructed with ICTP ranging from 0.01 to 250 $\mu\text{g/L}$ (7).

Mean ICTP concentration values were calculated in micrograms per liter and analyzed using analysis of variance and Student-Newman-Keuls multiple comparisons. Differences between storage times were analyzed by the Friedman and Wilcoxon pair-wise comparison tests. Significance was considered at $P < .05$.

Scanning Electron Microscopic/Energy-dispersive X-ray Analysis

Representative specimens of each group were fixed in a solution of 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer for 24 hours, rinsed 3 times in 0.1 mol/L sodium cacodylate buffer, and post-fixed in 1% osmium tetroxide solution for 2 hours. They were then rinsed in distilled water and dehydrated in an ascending ethanol series (30%, 50%, 70%, 80%, 95%, and 100%) for 15 minutes each. Samples were then critical point dried. Afterwards, they were sputter coated with gold by means of a sputter-coating Nanotech Polaron (Polaron Equipment Ltd, Watford, UK) and observed with a scanning electron microscope (S-510; Hitachi, Tokyo, Japan) at an accelerating voltage of 20 kV. Energy-dispersive analysis was performed using an x-ray detector system (EDX, Röntec, M-Series; Edwin, Berlin, Germany) attached to the Hitachi S-510 scanning electron microscope.

Results

ICTP Determination

Mean ICTP values were affected by the dentin treatment ($F = 211.50$, $P < .001$), infiltrated resin ($F = 761.45$, $P < .001$), and storage time ($F = 91.15$, $P < .001$). Interactions were also significant ($P < .001$). The reliability of the analysis of variance was 0.79. The mean and standard deviation of the ICTP concentration liberated from the dentin beams after each storage period is listed in Table 1.

If demineralization processes are compared, the amount of collagen degradation was significantly higher in the PA-demineralized dentin. Similar values were attained when dentin was EDTA treated. Collagen degradation was highly reduced when PA-etched dentin was resin infiltrated, and the same trend was observed in EDTA-treated dentin.

TABLE 1. Mean and Standard Deviation of ICTP Concentrations ($\mu\text{g/L}$) in Artificial Saliva after Human Dentin Beam Storage in the Different Experimental Groups

	Dentin State	24 h	1 wk	4 wk
No resin	Untreated	3.4 (0.42) A2	7.8 (0.75) B2	10.6 (1.83) C3
	PA	58.8 (6.31) A3	200.7 (10.10) B3	206.2 (8.69) B4
	EDTA	67.8 (7.29) A3	200.5 (7.42) B3	205.3 (8.77) B4
Resin	Untreated	1.2 (0.05) A1,2	1.8 (1.00) A1	3.2 (0.70) B2
	PA	3.3 (0.68) A2	6.7 (0.90) B2	14.5 (1.32) C3
	EDTA	3.5 (0.69) A2	6.7 (0.43) B2	17.0 (1.84) C3
Resin HCAT- β	Untreated	0.4 (0.10) A1	0.7 (0.79) B1	0.8 (0.06) B1
	PA	3.1 (0.73) A2	9.2 (0.83) B2	13.3 (0.90) C3
	EDTA	1.7 (0.34) A1,2	4.6 (0.65) B2	3.9 (1.75) B2
Resin BAG	Untreated	0.3 (0.09) A1	0.7 (0.07) A1	0.9 (0.04) A1
	PA	0.6 (0.06) A1	1.0 (0.06) A1	1.0 (0.14) A1
	EDTA	0.7 (0.07) A1	0.9 (0.15) A1	1.1 (0.22) A1

For each horizontal row, values with identical letters indicate no significant difference using the Friedman and Wilcoxon pair-wise comparisons tests ($P > .05$). For each vertical column, values with identical numbers indicate no significant difference between dentin treatments using the Student-Newman-Keuls test ($P > .05$).

The highest decrease in MMP-mediated collagen degradation was found when infiltrated resin was filled with BAG particles in both PA-demineralized and EDTA-treated dentin at the 4-week evaluation. When resin was filled with HCAT- β particles, collagen degradation was diminished only in untreated or EDTA-treated dentin. When untreated dentin was analyzed, ICTP values at 4 weeks followed the following trend: BAG = HCAT- β < unfilled resin < no resin.

Scanning Electron Microscopic/Energy-dispersive X-ray Analysis

Representative images from resin-infiltrated dentin surfaces after 4 weeks of water storage in artificial saliva are presented in Figures 1 and 2. In all groups, some resin was hydrolyzed, and dentin surfaces appeared just partially covered by resin. In the specimens bonded with neat resin (not filled) demineralized collagen with no clear sign of mineral (calcium/phosphate [Ca/P]) precipitation, some resin tags remained in the dentinal tubules (Figs. 1A and 2A). The specimens infiltrated with BAG-filled resin showed a calcium phosphate layer (clear Ca/P presence is detected by energy-dispersive x-ray analysis [Fig. 1B]) and mineral precipitates embedded in the collagen network (Figs. 1B and 2B). When dentin was PA etched and infiltrated with the HCAT- β -filled resin, only spot-distributed clusters of microglobular crystals were found (Fig. 1C). Conversely, in EDTA-treated dentin specimens infiltrated with the HCAT- β -filled resin, only a thin Ca/P layer was observed after 4 weeks of artificial saliva storage (Fig. 2C).

Discussion

The null hypothesis has to be rejected because MMP-mediated dentin collagen degradation decreased when demineralized dentin was infiltrated using resin filled with bioactive particles. The ICTP is the carboxy-terminal telopeptide region of type I collagen joined via trivalent cross-links and liberated during collagen degradation. The telopeptide is only produced through the action of MMPs. The determination of ICTP is one of the most reliable techniques to quantify enzymatic activity of MMPs on type I collagen (13).

Resin filled with BAG particles strongly inhibited MMP-mediated collagen degradation in all dentin demineralization states. BAG is a commercial calcium/sodium phosphosilicate composed of SiO_2 (45 wt%), Na_2O (24.5 wt%), CaO (24.5 wt%), and P_2O_5 (6 wt%) and binds with bone and soft tissues (14). It seems that there are 2 key composition features of BAG:

1. A content of around 60 mol% SiO_2 . There is evidence from *in vivo* studies that Si plays an important role in bone metabolism and collagen synthesis (15, 16).
2. A high $\text{CaO/P}_2\text{O}_5$ ratio, which makes BAG highly reactive to an aqueous medium and bioactive (14, 17). The high ratio of calcium to phosphorus has been shown to promote the formation of apatite crystals. Calcium and silica ions can act as crystallization nuclei (18). Other BAG formulations with lower Ca/P ratios do not bond to bone (14).

BAG is able to induce the up-regulation of MMP-2 and MMP-14 (2.7-fold and 3.6-fold, respectively). Additionally, 2 of their inhibitors, TIMP-1 and TIMP-2, were induced 2.2-fold and 2.1-fold, respectively. The induction of both metalloproteases and their inhibitors indicates an increase of extracellular matrix turnover (19) and a decrease in MMP-2 expression (20). The reduction of MMP-1 and MMP-2 has been shown to have a positive effect on bone formation (21).

Dentin sticks that were resin infiltrated with BAG-filled resin attained remineralization after 4 weeks (Figs. 1B and 2B). It is well established that an initial step in the conversion of silicate bioactive glass (such as 45S5) to hydroxyapatite (HA) is the formation of a SiO_2 -rich gel layer on the glass surface by ion exchange reactions. The dissolution of Ca^{2+} ions from the glass, their diffusion through the SiO_2 -rich layer, and their reaction with $(\text{PO}_4)_3^-$ ions from the solution lead to the precipitation of an amorphous calcium phosphate layer on the SiO_2 -rich layer, which subsequently converts to HA. The reaction interface moves inward with time (22). However, the precise role of Si in collagen and bone homeostasis remains unknown (17). A cellular receptor for Si has not been identified, and the precise role of Si in hard-tissue homeostasis is unclear (17).

It seems that ionic dissolution products from these inorganic materials when exposed to biological fluids are responsible for their biological activity. Ionic dissolution products released from BAG have been shown to activate alkaline phosphatase, to diminish scaffolds degradation, and to increase collagen production in tissues, enhancing extracellular matrix deposition and mineralization (6). In summary, it stimulates a path of regeneration and tissue self-repair processes. Bioactive glasses produced a well-characterized ion exchange mechanism occurring at the interface of the material with biological simulated solutions (6). The process of ion exchange is followed by network dissolution. Analysis of BAG-conditioned medium showed an 88-fold higher Si concentration and, to a much lesser extent, changes in Ca and P concentrations relative to other tested control materials (17). However, it still remains unclear which ionic products (and in which concentration) are

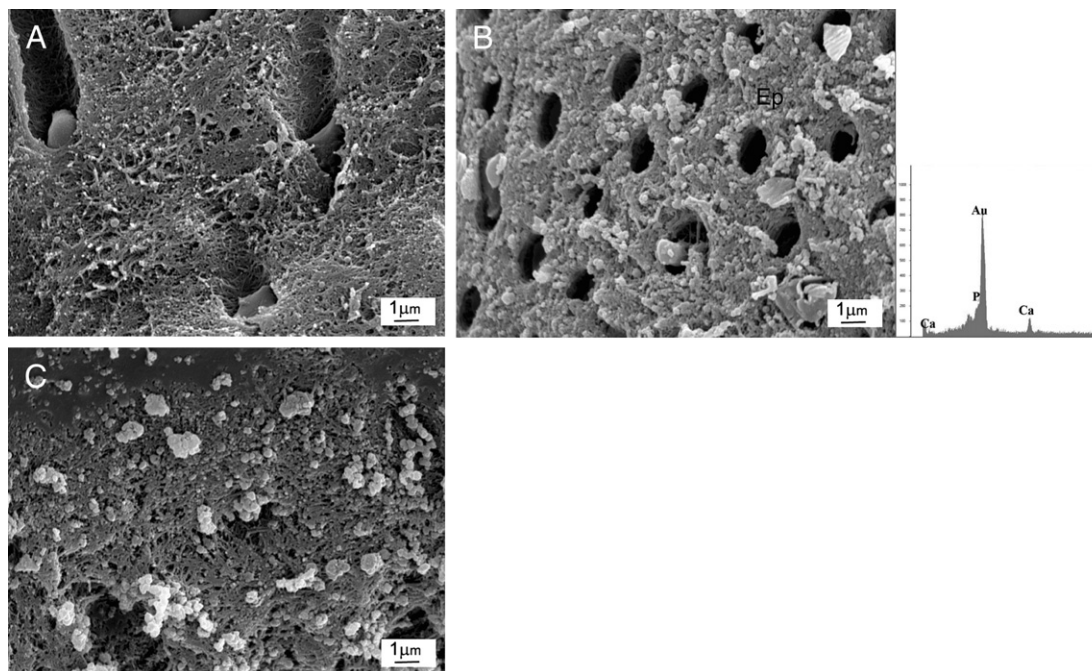


Figure 1. Scanning electron microscopic pictures of dentin surfaces after 4 weeks of artificial saliva storage. Dentin was PA etched and infiltrated with (A) neat resin (demineralized collagen with no clear sign of mineral precipitation is observed, some resin tags remained in the dentinal tubules); (B) resin filled with BAG particles (energy-dispersive x-ray spectra included), a Ca/P layer (clear Ca/P presence is detected by energy-dispersive x-ray analysis), and mineral precipitates embedded in the collagen network; and (C) resin filled with HCAT- β particles (only spot-distributed clusters of microglobular crystals were found).

responsible for repair processes activation and which are the exact mechanisms of interaction with the ionic products. However, it seems that the Si concentration and the Ca/P ratio are critical.

When CaP formation is produced, MMP-2 and MMP-9 form complexes (CaP:MMP) with high molecular weight and restricted mobility (23). Amorphous CaP is a precursor to more crystalline forms

of CaP such as HA. Interestingly, it has also been reported that prolonged HA (>4 hours) induces the inactivation of some MMPs (24). Both facts may help to explain the extremely low ICTP values attained for these resin-infiltrated and remineralized dentin beams.

A similar mechanism of action to produce MMP inhibition may be responsible for the results observed in the specimens infiltrated with

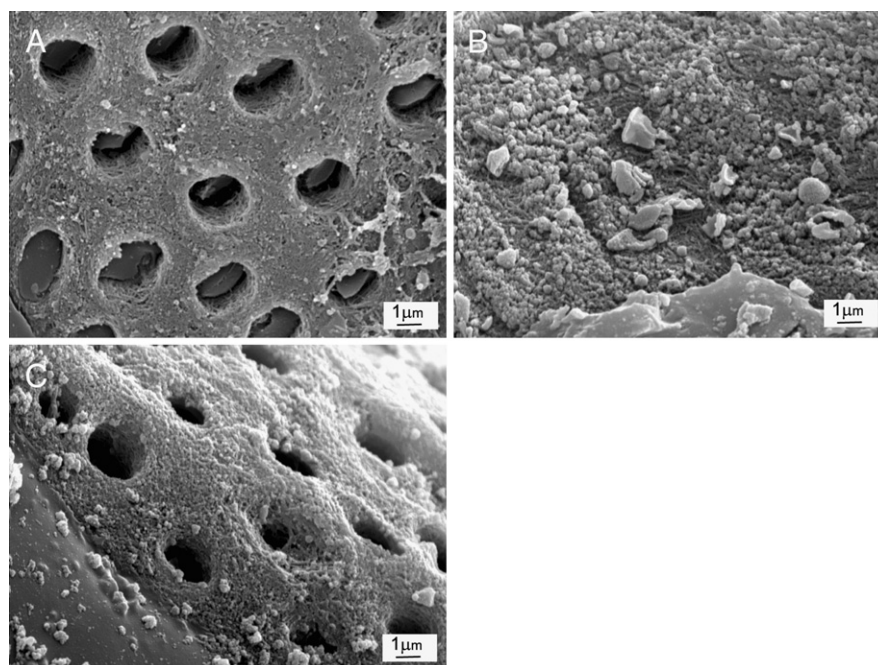


Figure 2. Scanning electron microscopic images of dentin surfaces after 4 weeks of artificial saliva storage. Dentin was EDTA treated and infiltrated with (A) neat resin (demineralized collagen with no clear sign of mineral precipitation is observed, some resin tags are patent), (B) resin filled with BAG (particles and mineral precipitates embedded in the collagen network are evidenced), and (C) resin filled with HCAT- β particles (a thin Ca/P layer may be observed).

HCA- β particles. Hexacalcium aluminate trisulfate (known as ettringite) is formed during the setting reaction of Portland cement. It sets in contact with water and undergoes a hydration process in which Ca^{2+} and OH^- ions are released from tricalcium silicate (C_3S) into the surrounding environment. At supersaturation levels, it forms calcium hydroxide (portlandite) precipitate and amorphous calcium silicate hydrate gel (25). Therefore, there is a great availability of silica in this type of filler because it comes from the polymerization of the silicate component and the formation of the C-S-H phase after cement setting (26, 27). Our HCA- β filler also has a great source of OH and calcium. Calcium release from calcium silicate particles and the formation of Si-OH groups has been shown to facilitate the formation of an apatite layer (26). It is also known that Si-OH groups are catalyzing agents providing favorable sites for HA nucleation (26). However, low MMP activity was only found when dentin was mineralized or EDTA treated. It may be explained by the phosphorous content in HCA- β because it is lower than that of the BAG particles. A critical phosphorous and Si concentration is essential for calcium phosphate deposition and extracellular matrix mineralization. It is clear that phosphorus contained in PA-treated dentin is lower than that of mineralized or EDTA-treated dentin. EDTA does not completely remove noncollagenous proteins from dentin so phosphoproteins are present (28). The role of phosphate in the aiding of nucleation of apatite on a biological surface can be played by phosphate ions existing adsorbed on the organism itself (18).

It has also been shown that other calcium silicate cements possess bioactivity when immersed in phosphate-rich buffered solutions (simulated body fluid) and are able to induce the formation of apatite precipitates (10, 11). In low-concentration phosphate solutions (Hank's solution), a reduced rate of apatite precipitation and an increase in the time required for superficial apatite formation were found (27). In our material design, among various calcium phosphate phases, β -TCP was selected because it is biocompatible and resorbable (29). However, even when β -TCP was mixed with the calcium silicate cement in the experimental formulation, as an active compound providing phosphate to enhance apatite formation, it may be that the phosphate concentration was not enough to induce rapid remineralization in PA-treated dentin because of the low dissolution rate of β -TCP in the alkaline environment (29). The ionic composition of such biomaterials is a key factor in their bioactivity.

Surface precipitates embedded in the protected collagen network were evident in the specimens infiltrated with BAG-filled resin after 4 weeks of immersion in artificial saliva (Figs. 1B and 2B). However, in the dentin specimens infiltrated with the HCA- β -filled resin, only spot-distributed clusters of microglobular crystals were found (Fig. 1C). These morphologic features strongly suggest the higher bioactivity of BAG-filled resin. Mineral precipitation was produced in artificial saliva (nonphosphate solution) and was facilitated by ionic dissolution (calcium and phosphate) released by the experimental bioactive-filled blends. Phosphorylation of dentin collagen has previously been shown to cause a significant surface remineralization of partially demineralized dentin (30).

HCA- β particles could probably be improved by increasing the phosphorous content. As has been shown, even when the Ca/Si availability in set calcium silicate cements is very high and it increases over time, the bioactive behavior of calcium silicate materials is affected by the presence of phosphorus, which can lead to a major rate of formation of the apatite phase (27).

However, when dentin was EDTA treated, the bioactivity of resin filled with HCA- β particles was augmented because ICTP values were lower and remineralization was evident in the scanning electron microscopic images (Fig. 2C). These results may be explained by a biological interaction between EDTA-treated dentin and β -TCP particles. It

has been shown that an experimental graft material based in β -TCP was able to bond to EDTA-treated dentin because β -TCP particles clung to the EDTA-treated dentin surface and acted as nucleation sites to adsorb phosphate and calcium, finally forming apatite particles (31). Pinna et al (32) also described better dentin sealing when a calcium silicate resin-based cement modified with α -TCP was applied to EDTA-demineralized dentin. To explain this specific chemical interaction, it has been found that collagen molecules in β -TCP/collagen composite showed higher intermolecular assembly and covalent bonding between the Ca^{++} and RCOO^- ions of the collagen fibers than that of HA/collagen. These reactions were greatly increased by the catalysis of polyphosphate generated during mixing (33).

BAG particles have been proposed as bioactive filler components and MMP inhibitors to be included in resin-based cements. High bioactivity is the main advantage of BAG. The persistence of bioactive ionic availability of this mixture was shown by the action of this material on dentin MMPs and its rapid dentin remineralization induction. Phosphorylated extracellular proteins play critical roles in the formation of collagenous mineralized tissues and in the regulation of biomineralization (34). However, it has been shown that phosphoproteins are nonspecific and may be substituted by other immobilized, naturally occurring phosphoproteins to induce apatite deposition within collagen (35). When bound to collagen, ions from these BAG particles are conjectured to perform a templating function resulting in remineralization of demineralized collagen fibrils.

This approach to investigate the major molecules involved in polymer-tissue interface degradation may provide sensitive indicators of tissue remodeling at the tissue-biomaterial interface. However, the highest challenge for developing dentin remineralization through bioactive-filled resins is to achieve the controlled dissolution and ion release kinetics, making sure that the required critical concentrations are obtained. Designed experimental cements possess high *in vitro* bioactivity, limiting dentin MMP collagenolytic action and favoring remineralization of EDTA-treated dentin and should be considered as promising root-filling cements.

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The authors deny any conflicts of interest related to this study.

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